

CLAIMS

What is claimed is:

1. A carotenoid overproducing bacteria comprising the genes encoding a functional carotenoid enzymatic biosynthetic pathway wherein the *dxs*, *idi* and *ygbBP* genes are overexpressed and wherein the *yjeR* gene is down regulated.
- 5 2. A carotenoid overproducing bacteria comprising the genes encoding a functional carotenoid enzymatic biosynthetic pathway wherein the *dxs*, *idi*, *ygbBP* and *ispB* genes are overexpressed.
- 10 3. The carotenoid overproducing bacteria of Claim 1 or 2 wherein the *lytB* and *dxr* gene is optionally overexpressed.  
*ispB* *lytB* and *dxr* *yjeR*
- 15 4. The carotenoid overproducing bacteria of Claim 1 or 2 wherein the carotenoid enzymatic biosynthetic pathway consists of the genes *dxs*, *dxr*, *ygpP*, *ychB*, *ygbB*, *lytB*, *idi*, *ispA*, *ispB* *crtE*, *crtB*, *crtI*, and *crtY*.
5. The carotenoid overproducing bacteria of Claim 4 wherein the carotenoid enzymatic biosynthetic pathway optionally additionally comprises the *crtZ* and *crtW* genes.
- 20 6. The carotenoid overproducing bacteria of any of Claims 1-5 wherein the bacteria is selected from the group consisting *Agrobacterium*, *Erythrobacter*, *Chlorobium*, *Chromatium*, *Flavobacterium*, *Cytophaga*, *Rhodobacter*, *Rhodococcus*, *Streptomyces*, *Brevibacterium*, *Corynebacteria*, *Mycobacterium*, *Deinococcus*, *Paracoccus*, *Escherichia*, *Bacillus*, *Myxococcus*, *Salmonella*, *Yersinia*, *Erwinia*, *Pantoea*,  
25 *Pseudomonas*, *Sphingomonas*, *Methylomonas*, *Methylobacter*, *Methylococcus*, *Methylosinus*, *Methylomicrobium*, *Methylocystis*, *Alcaligenes*, *Synechocystis*, *Synechococcus*, *Anabaena*, *Thiobacillus*, *Methanobacterium*, *Klebsiella*, and *Myxococcus*.
- 30 7. The carotenoid overproducing bacteria of Claim 6 wherein the bacteria is *E. coli*.
8. The carotenoid overproducing bacteria of Claims 1-3 wherein the *dxs*, *dxr*, *ygpP*, *ychB*, *ygbB*, *lytB*, *idi*, *ispA*, *ispB* are derived from a *Methylomonas* sp..
- 35 9. The carotenoid overproducing bacteria of any of Claims 1 – 3 wherein the *dxs*, *idi*, *ispB* and *ygbBP* genes are under the control of a strong promoter.

10. The carotenoid overproducing bacteria of Claim 9 wherein the strong promoter is selected from the group consisting of *lac*, *ara*, *tet*, *trp*,  $\lambda P_L$ ,  $\lambda P_R$ , *T7*, *tac*,  $P_{T5}$ , and *trc*.

11. The carotenoid overproducing bacteria of any of Claims 1-3  
5 wherein the *dxs*, *idi*, *ispB* and *ygbBP* genes are integrated in multicopy in the bacterial chromosome.

12. The carotenoid overproducing bacteria of any of Claims 1-3 wherein the *dxs*, *idi*, *ispB* and *ygbBP* genes are present in multicopy in the bacteria on one or more plasmids.

10 13. The carotenoid overproducing bacteria of of Claim 7 wherein the *yjeR* gene is down regulated by gene disruption.

14. The carotenoid overproducing bacteria of Claim 13 wherein the disrupted *yjeR* gene has the nucleotide sequence as set forth in SEQ ID NO:63.

15 15. The carotenoid overproducing bacteria of either of any of Claims 1 –3 wherein the *dxs*, *idi*, *ispB* *ygbBP* and *lytB* genes are chromosomally integrated into the host cell genome.

20 16. A carotenoid overproducing bacteria selected from the group consisting of: a strain having the ATCC identification number PTA-4807 and a strain having the ATCC identification number PTA-4823.

17. A method for the production of a carotenoid comprising:  
25 a) growing the carotenoid overproducing bacteria of any of Claims 1 –5, the bacteria overexpressing at least one gene selected from the group consisting of *dxs*, *idi* *ygbBP*, *ispB*, *lytB*, *dxr*, wherein *yjeR* is optionally downregulated, for a time sufficient to produce a carotenoid; and  
b) optionally recovering the carotenoid from the carotenoid overproducing bacteria of step (a).

30 18. A method according to Claim 17 wherein the carotenoid is selected from the group consisting of antheraxanthin, adonixanthin, astaxanthin, canthaxanthin, capsorubrin,  $\beta$ -cryptoxanthin, didehydrolycopene, didehydrolycopene,  $\beta$ -carotene,  $\zeta$ -carotene,  $\delta$ -carotene,  $\gamma$ -carotene, keto- $\gamma$ -carotene,  $\psi$ -carotene,  $\varepsilon$ -carotene,  $\beta,\psi$ -carotene, torulene, 35 echinenone, gamma-carotene, zeta-carotene, alpha-cryptoxanthin, diatoxanthin, 7,8-didehydroastaxanthin, fucoxanthin, fucoxanthinol, isorenieratene,  $\beta$ -isorenieratene lactucaxanthin, lutein, lycopene, neoxanthin, neurosporene, hydroxyneurosporene, peridinin, phytoene,

rhodopin, rhodopin glucoside, siphonaxanthin, spheroidene, spheroidenone, spirilloxanthin, uriolide, uriolide acetate, violaxanthin, zeaxanthin- $\beta$ -diglucoside, zeaxanthin, and C30-carotenoids.

19. A method according to Claim 18 wherein the carotenoid is  
5 produced at a level of at least about 6 mg per gram dry cell weight.

20. A method according to Claim 18 wherein the bacteria is selected from the group consisting *Agrobacterium*, *Erythrobacter*, *Chlorobium*, *Chromatium*, *Flavobacterium*, *Cytophaga*, *Rhodobacter*, *Rhodococcus*, *Streptomyces*, *Brevibacterium*, *Corynebacteria*,  
10 *Mycobacterium*, *Deinococcus*, *Paracoccus*, *Escherichia*, *Bacillus*, *Myxococcus*, *Salmonella*, *Yersinia*, *Erwinia*, *Pantoea*, *Pseudomonas*, *Sphingomonas*, *Methylomonas*, *Methylobacter*, *Methylococcus*, *Methylosinus*, *Methylomicrobium*, *Methylocystis*, *Alcaligenes*, *Synechocystis*, *Synechococcus*, *Anabaena*, *Thiobacillus*,  
15 *Methanobacterium*, *Klebsiella*, and *Myxococcus*.

21. A method according to Claim 20 wherein the bacteria is *E. coli*.  
22. A method according to Claim 17 wherein the *dxs*, *idi*, *ygbBP*, *ispB* and *lytB* genes are under the control of a promoter selected from the group consisting of *lac*, *ara*, *tet*, *trp*,  $\lambda P_L$ ,  $\lambda P_R$ , *T7*, *tac*, *P<sub>T5</sub>*, and *trc*.  
20 23. A method according to Claim 17 wherein the *dxs*, *idi*, *ispB*, *ygbBP* and *lytB* genes are integrated in multicopy in the bacterial chromosome.

24. A method according to Claim 17 wherein the *dxs*, *idi*, *ispB*, *ygbBP* and *lytB* genes are in multicopy in the bacteria on one or more plasmids.  
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25. A method according to Claim 17 wherein the *yjeR* gene is down regulated by gene disruption.

26. A method according to Claim 25 wherein the disrupted *yjeR* gene has the nucleotide sequence as set forth in SEQ ID NO:63.  
30 27. A method according to Claim 17 wherein the *dxs*, *idi* *ispB*, *ygbBP* and *lytB* genes are chromosomally integrated into the host cell genome.